Separation of non-sedimental carbon dots using “hydrophilicity gradient ultracentrifugation” for photoluminescence investigation

Li Deng, Xiaolei Wang, Yun Kuang, Cheng Wang, Liang Luo (✉), Fang Wang, and Xiaoming Sun (✉)

Nano Res., Just Accepted Manuscript • DOI 10.1007/s12274-015-0786-y
http://www.thenanoresearch.com on April 8, 2015

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A “hydrophilicity gradient ultracentrifugation” method to sort non-sedimental carbon nanodots (CDs) is established. The CDs were pre-treated by acetone to form clusters and then “de-clustered” as they were forced to sediment through the media according to the hydrophilicity difference.

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Separation of non-sedimental carbon dots using “hydrophilicity gradient ultracentrifugation” for photoluminescence investigation

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Received: day month year
Revised: day month year
Accepted: day month year (automatically inserted by the publisher)

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KEYWORDS
Nanoseparation, Carbon dots, Hydrophilicity gradient, Pre-aggregation, De-clustering, Photoluminescence mechanism

ABSTRACT
Carbon nanodots (CDs) made by hydrothermal dehydration were always a mixture of nanoparticles with various size and carbonized degree while common ultracentrifugation failed to sort them due to the extremely high colloidal stability. Here we introduce a hydrophilicity gradient ultracentrifugation method to sort such non-sedimental CDs. The CDs synthesized from citric acid and ethylenediamine were pre-treated by acetone to form clusters. Such clusters “de-clustered” as they were forced to sediment through the gradient of ethanol and water with varied volume ratios. The primary CDs with varied size and carbonized degree detached from the clusters and were well dispersed in the corresponding gradient layers, which was highly dependent on the environmental media with varied hydrophilicity/solubility. Thus, the proposed hydrophilicity triggered strategy could be used for sorting nanoparticles with extremely high colloidal stability, which further widened the range of sortable nanoparticles. More importantly, according to careful analysis on the change in size, composition, quantum yield and transient fluorescence of typical fractions after separation, it is concluded that the photoluminescence of as-prepared hydrothermal carbonized CDs mainly arose from the surface molecular state rather than size difference.

1 Introduction
After being accidently discovered during the electrophoretic purification of single-wall carbon nanotubes (SWCNT) [1], carbon nanodots (CDs) have gradually become a novel rising star as a member of carbon nanomaterials [2-4]. CDs have been found to be sp² character as the symbolic of nanocrystalline graphite [2]. Compared with bulk materials, due to the size below 10 nm, CDs exhibit strong quantum effect, and consequently fascinating unique optical properties. The high photoluminescence quantum yield, high photostability, anti-photobleaching, and especially the low-toxicity/good biocompatibility and non-blinking other than semiconductor QDs, make CDs an excellent candidate for various applications such bioimaging, sensing and optoelectronics [5-10]. In this regard, a tremendous amount of research effort has been devoted to photoluminescence...
mechanism investigation and synthesis method optimization. Two main groups have been proposed for low cost and large-scale production: physical and chemical methods (or “top-down” and “bottom-up”). Physical methods include arc discharge, laser ablation, and plasma treatment [1, 11, 12]. Chemical methods include combustion/thermal/hydrothermal oxidation, microwave/ultrasonic, confined synthesis and so on [10, 13-15]. For the practical bio-applications, facile and economic manipulation of size, chemical composition, and surface properties is of particular importance, and thereby the hydrothermal or microwave reaction is the designable and attractive choice. Molecules or polymers with multiple hydroxyl, carboxyl and amine groups, such as citric acid and amines, were chosen as starting materials. Recently, Yang et al reported a facile hydrothermal carbonization method based on citric acid and ethylenediamine for the fabrication of fluorescent CDs with quantum yield as high as 80%. Such thermal carbonized CDs showed strong blue photoluminescence (PL). Considering the complex and diverse structural characters among CDs, the origins of PL are still under active debate, such as the quantum size effect [7], triplet carbenes at the zigzag edges [16, 17], radiative recombination of the excitons [2, 18] and the molecular state (as for organic dyes). Therefore, definite experimental evidence for the PL property involved with size effect still awaits further investigation, especially the effective nano-separation of CDs.

In recent years, the density gradient ultracentrifuge rate separation (DGURS) method has emerged as an efficient way of sorting nanoparticles of different sizes, compositions and morphologies [19-21]. This strategy has also provided new opportunity to isolate/capture intermediate product for the observation and understanding of chemical reaction, growth process or phase transition [22-24]. However, as we extended this method to separate CDs, it was found that the CDs were too hard to sedimentate. Because, CDs contain many carboxylic acid moieties at their surface and subsequently strong hydrogen bonding between CDs and water is induced, which endowed them with too good water-solubility to sedimentate. This makes the obstacle for consequent separation.

Herein, we designed a “hydrophilicity gradient ultracentrifuge” separation technique based on solubility or colloidal stability difference of CDs in different media to separate CDs. The CDs were synthesized from citric acid and ethylenediamine, and pre-treated by acetone to form clusters. Solutions of water in ethanol with different volume ratios were used as the separation layer gradient, which could provide not only the density difference but more importantly the hydrophilicity difference to catch CDs with specific solubility. During the subsequent separation process, the pre-aggregated CDs were separated with different carbonized degree and size in one tube depending on hydrophilicity difference of fractions. The proposed technique offers an effective strategy for the separation of species which have strong interactions with solvents and hard to sedimentate. This work is also important for the understanding of photoluminescence mechanism of CDs. In particular, for each fraction we analyzed the change in size, composition, quantum yield and transient photoluminescence, and the analysis provided solid evidence that the photoluminescence of as-prepared thermal carbonized CDs mainly arose from the radiative recombination of excitons related to the surface chemical groups rather than size difference.

2 Experimental
2.1 Reagents:
Citric acid and ethylenediamine were purchased from SIGMA-ALDRICH. Ethanol and acetone were purchased from Beijing Chemical Reagents Company. All the reagents were used without any purification and deionized (DI) water was used in all experiments.

2.2 Preparation of carbon dots (CDs):
CDs were synthesized by a hydrothermal treatment of citric acid and ethylenediamine [10]. The detailed method was first dissolving citric acid (1.75 g) and ethylenediamine (560 μL) into deionized water (16 mL). Then the solution was transferred to a poly(tetrafluoroethylene) (Teflon)-lined autoclave (50 mL) and heated at 200 ºC for 5 h. After the reaction, the reactors were cooled to room temperature by water or naturally.

2.3 Multifunctional gradient preparation:
The separation layer step gradient was made using 20%, 30%, 40% and 50% (by volume) solutions of
water in ethanol. For instance, a volume ratio of water: ethanol = 2:8 was used to make the 20% solution. A step gradient was created directly in Beckman centrifuge tubes (polyallomer) by adding layers to the tube with decreasing water concentration. To make a (20% + 30% + 40% + 50%) gradient, 0.8 mL of 50% solutions of H2O in ethanol was first added to the centrifuge tube, then 0.8 mL 40% solutions of H2O in ethanol was slowly layered above the 50% layer. The subsequent layers were made following the same procedure and resulted in a hydrophilicity as well as density gradient along the centrifuge tube.

2.4 Typical centrifugation of CDs:
The prepared colloidal solution of CDs was pretreated by first diluting to double of volume with deionized water and then adding 20 fold volume of acetone to the diluted solution forming a seriflux containing CDs clusters. Layered 0.8 mL of the seriflux slowly above the density gradient and centrifuged at 50,000 rpm for 12 h. After the centrifugation, samples were pipetted gently for further characterization. Each sample contained 200 μL liquid (fraction 1–fraction 20, briefly, f1–f20). Precipitations at the bottom of the centrifuge tube were resuspended into deionized water through ultrasonic processing which formed f21. In order to facilitate subsequent tests, all the fractions were evaporated to remove the organic solvent, and then redissolved into deionized water. Only typical fractions were picked out to detect optical properties and structure in order to study the separation principle as well as influential factor of CDs FL intensity.

2.5 Characterization:
The optical absorption of the CDs was measured by UV-vis spectroscopy (UV-2501PC, Shimadzu, working in the range 300–1100 nm), and the PL emission spectra was measured at 340nm excitation wavelength on Hitachi F-7000 photoluminescence spectrometer. The sizes of the CDs were measured under high-resolution transmission electron microscopy (HRTEM; JEOL, JEM-2100, 200 kv). FTIR spectra were measured on Nicolet 6700 FTIR spectrometer. XPS measurements were performed using a Thermo Electron ESCALAB 250 instrument with Al Kα radiation. Raman spectra were recorded on the Lab RAM ARAMIS Raman system with a 633 nm argon ion laser as excitation. The fluorescence decay of carbon dots was measured by the picosecond time-resolved fluorescence spectrometer, using a Ti:sapphire regenerative amplifier (Spitfire, Spectra Physics) as the excitation pulses. The excitation pulse energy was ~100 nJ/pulse at a pulse repetition rate of 1 kHz which was focused onto a spot with diameter less than 0.5 mm. Photoluminescence (PL) collected with the 90 degree geometry was dispersed by a polychromator (250is, Chromex) and collected with a photon-counting type streak camera (C5680, Hamamatsu Photonics). The data detected by digital camera (C4742-95, Hamamatsu) were routinely transferred to computer for analysis with HPDTA software. The spectral resolution was 2 nm, and the temporal resolution was 2–100 ps, depending on the delay-time-range setting.

3 Results and discussion

Properties of original CDs
The CDs were prepared by hydrothermal treatment of solution of citric acid and ethylenediamine at 200 °C [10]. The as-prepared product was brownish solution after dilution and exhibited strong blue photoluminescence under UV light (Fig. 1a inset). The absorption spectrum of the as-prepared CDs had a typical peak at 340 nm and when they were excited at 340 nm, a maximum emission peak at 440 nm was observed (Fig. 1b). According to high resolution transmission electron microscopy (HRTEM, Fig. 1a) image, most particles were found to be amorphous structure without lattices. Only a small amount of particles (<2%) showed well-resolved lattice fringes with lattice spacing of 0.21, 0.26 and 0.33 nm, respectively, which were in accord with the (100), (020) and (002) facets of graphite carbon (Fig. S1 in the Electronic Supplementary Material (ESM)). The statistic data of diameter distribution (Fig. 1c) showed that the size of CDs ranged from 0.6 to 11.8 nm, which indicated that the as-prepared CDs were not monodisperse owing to the inevitable heterogeneity of the hydrothermal reaction. Hence, effective nano-separation of CDs is required to investigate the photoluminescence mechanism.
Figure 1. (a) HRTEM image of as-prepared CDs, inset: digital camera images under ambient light (left) and UV light (right). (b) UV/Vis absorption (black), PL excitation (blue), and emission (red, excited at 340 nm) spectra of CDs. (c) Size distribution of CDs.

However, the as-prepared CDs were very stable in water, and they could not be centrifuged down from water even with 90,000 rpm for 10 h (Fig. S2 in the ESM). CsCl solution was also tried to change the dispersity of CDs in water (“salt effect”, Fig. S3 in the ESM), but CDs were still unable to be separated effectively even after 36 h under 50,000 rpm, confirming the good tolerance of CDs for physical salt concentration in practical applications [10]. Hence, we changed the solvent types to adjust the dispersity of CDs during the separation. Alcohols, as the common solvents, were introduced owing to good miscibility with water, weaker polarity, less hydroxyl ratio (weaker hydrogen bonding) and lower density than water. The same amount of diluted aqueous CDs solutions were diluted by methanol, ethanol, isopropanol, and water (as control); after low speed centrifugation as 12,000 rpm for 10 min, there was no precipitation in water while different amount of brown precipitates were observed in three alcohol-containing tubes (precipitate amount: methanol < ethanol < isopropanol, Fig. 2a). This verified that the hydrophilicity difference, or hydroxyl ratio of solvent molecules significantly affected the dispersity/sedimentation of CDs, which was the key point for the separation.

Separation of CDs using hydrophilicity gradient ultracentrifugation

In light of these observations, we redesigned a new separation method, hydrophilicity gradient ultracentrifugation, for separating CDs (Fig. 2b). For the fine control of CDs separation, ethanol was chosen as the mixed solvent with water due to the moderate hydroxyl ratio. A four-layer gradient medium involved density/hydrophilicity was made with 20%, 30%, 40% and 50% H2O/ethanol solutions.

To make a stable gradient, the top layer containing CDs for separation should have lower density than any layer in gradient. But at the same time, it should keep relatively high CDs concentration to meet the consequent characterization requirements. Ethanol was tried as the top medium, but the very fast and heavy aggregation and self-sedimentation action were observed even without centrifugation, possibly due to the too high concentration of CDs (Fig. S4 in the ESM, the solution in Fig. 2a was after 50 times dilution). As diluted by acetone with various volumes, there were stable suspensions obtained even for the very high concentrated aqueous solution of CDs (Fig. S5 in the ESM), possibly due to the formation of “water-in-oil like” clusters of CDs in the water/acetone mixed solutions [25, 26], which further increased the amount of target sample and provided a chance to improve the separation efficiency. The subsequent detaching degree of fractions from the clusters is very important for the separation effect, and is closely related to the formation of the initial aggregation, which is indeed determined by the volume-ratio-induced size of clusters. For the relative high concentrated clusters (20 folds of acetone without the two folds of water dilution), the QY measurement implied that there was obvious data jitter for the bottom fractions (Fig. S6). While for the much diluted concentration of clusters (20 folds of acetone and 100 folds of water), we can clearly see that the fractions could only pass through very limited layers after centrifugation (Fig. S7), namely, incomplete separation. Hence, during the centrifugation, there was a dynamic balance between sedimentation and detaching for the clusters, which would prevented some fractions with high hydrophilicity releasing duly under too high concentration of clusters, or incomplete separation under too diluted concentration. After repeated test, the optimized condition to make the samples for separation was: two folds of water was added into each as-prepared CDs suspension, diluted by 20 folds acetone to form homogeneous slurry, which was layered on top of the gradient medium.
The centrifugation was performed at 50,000 rpm for 12 h. After the centrifugation, the CDs were separated along the centrifuge tubes. Under 365 nm UV light irradiation, the photoluminescence intensity of the fractions in the centrifuge tube varied regularly from top to bottom. Fig. 2d shows the UV-vis spectra of typical fractions from top to bottom in centrifuge tube and the original unseparated sample. There was no obvious absorption peak-shift for the separated fractions, except very limited shift (<10 nm) for few bottom fractions such as f17, indicating essentially the same energy gap for each fraction. The centrifuge tube in Fig. 2b showed apparent color change possibly due to “inner filter effect” [27]. Correspondingly, the photoluminescence spectra of the separated fractions (Fig. 2e) with excitation at 340 nm showed no significant peak position difference, but the photoluminescence intensity of fractions monotonely decreased from top to bottom in the centrifuge tube, suggesting decreasing Quantum yield (QY) of fractions (Fig. 2f).

**Figure 2.** (a) Digital photographs of CDs solutions after centrifugation in water, methanol (MeOH), ethanol (EtOH) and isopropanol (IPA). (b) Digital photographs of centrifuge tube before (under ambient light) and after (left: under ambient light, and right: under UV light) centrifugation. (c) Scheme of proposed mechanism of separation: CDs clustered at starting point and de-clustered at succeeding layers with increased water ratio during sedimentation. (d) Normalized absorption and (e) photoluminescence spectra of original sample and separated fractions (excited at 340 nm, and all samples were diluted to the same absorbance). (f) Quantum yield variation of typical fractions, showing more stable CDs have higher fluorescence.

**HRTEM analysis**

The effectiveness of the “hydrophilicity gradient separation” and the carbonization difference were further confirmed by HRTEM (Fig. 3). Nine fractions including f1, f5, f9, f11, f13, f15, f17, f19 and f21 were chosen as the representative HRTEM samples, which were all nearly monodisperse (except f1 and f5). The top fractions, f1 and f5, were fish-scale-like polymer species with very weak contrast and blurry edges (Fig. S8 in the ESM), which were typically polymer-like dehydrated species at the very initial stage. Such species were very stable in ethanol-rich environment, suggesting the hydrophilicity similarity of such species with environments (Fig. 2b) and further confirmed the polymer-like nature. The QYs of f1 and f5 were steadily higher than 0.45,
implying the polymer-like species were very bright under UV-light irradiation, which was in accord with previous reports [28].

![Image](https://www.editorialmanager.com/nare/default.asp)

**Figure 3.** HRTEM images of typical fractions (from f9 to f21) and the statistics of size, inset: Magnified HRTEM images of typical particles, scar bars are 5 nm.

For the middle fractions (from f9 to f13), there were identifiable discrete nanoparticles with still low contrast but relatively clear edges, which implied increased condensing and carbonization degree. Their size was only 2-10 nm, much smaller than upper fractions (tens of nanometers), and interestingly bigger nanoparticles located at higher positions (size sequence: f9>f11>f13). This apparently abnormal phenomenon could be explained only based on hydrophilicity difference. Those big CDs reaching the media with similar hydrophilicity to ambient media would detach from clusters (de-cluster) and get stabilized therein without visible sedimentation. In contrast, the smaller particles in f13-21 had less hydrophilicity, and were more compatible with water-rich environment (bottom half). The QYs of these fractions further demonstrated their difference in nature besides solubility: It decreased dramatically from 0.48 (f9) to 0.05 (f13). We can infer that the abundant molecular state of polymer-like species might be the origin of fluorescence since f9 was still polymer-like, but f13 was basically more carbon-like.

For the bottom fractions (from f15 to f21) shown in Fig. 3, the contrast became higher, and edges turned clearer, and the average size became bigger, from 1.54 nm (f15) to 5.1 nm (f21). They should be the species after more complete carbonization, and the corresponding QYs of these fractions remained lower than 0.05 (Figure 2f). Size measurements were carried out on 100-200 CDs per sample (Fig. S9 in the ESM) and the average size of each fraction were summarized in the bottom right graph of Fig. 3. In detail, the average size of fractions from top (f9) to bottom (f21) in the centrifuge tube gradually decreased from 9.5 nm (f9) to 1.54 nm (f15), then increased to 5.1 nm (f21). Interestingly, different from classic result of DGURS, the smallest fractions (f15) located at the middle region of centrifuge tube rather than top region, which, as mentioned before, was because the separation of nanoparticles was based on hydrophilicity difference of CDs while the species with different hydrophilicity naturally have various sizes. Specifically, according to the HRTEM results, the size distribution of upper half fractions exhibited a decrease trend (Fig. 3); for the bottom half fractions,
the size distribution (from f15 to f21) exhibited an increase trend (Fig. 3); and all the separated fractions stopped sedimentation (Fig. 2c) after getting discrete in media with certain hydrophilicity even with elongated centrifugation time. Judging from the movement features, the separation process could be regarded as modified isopycnic separation. Importantly, the trend in fluorescence spectra and QYs indicated that the photoluminescence of CDs were not related to their size.

It is known that the carbonization degree in relationship of hydrothermal time. Elongated hydrothermal treatment naturally strengthen the condensation and carbonization process, therefore, we compared the PL spectra of above fractions with CDs prepared at varied hydrothermal treatment time (7, 10 and 20 h). Interestingly, they showed very high similarity in PL trend. It could be seen from Fig. S10 in the ESM that there was no peak shift but width broadening for absorption peaks and quantum yield decreasing with the elongated hydrothermal treatment, which was in accord with the trend of fractions in centrifuge tube (Fig. 2d and 2f). We could infer that the hydrophilicity gradient separation made fractions redistributed according to carbonization degree (low-to-high from top to bottom, Fig. 2c). Also, the QY decreased with increased carbonization degree, implying carbon did not promote the fluorescence.

**FTIR analysis**

To get deeper insight into the difference of fractions in chemistry and structure, Fourier Transform Infrared (FTIR) spectra were recorded (Fig. 4). The vibration intensity of functional groups of CDs [10] such as stretching vibrations of C-OH at 3430 cm⁻¹ and C-H at 2923 cm⁻¹ and 2850 cm⁻¹, asymmetric stretching vibrations of C-NH-C at 1126 cm⁻¹, bending vibrations of N-H at 1570 cm⁻¹, and the vibrational absorption band of C=O at 1635 cm⁻¹ regularly decreased as the fraction number increased from f1 to f17. The result suggested that there were richer functional groups on the fractions located near to the top, but CDs with less functional groups would sediment down to the bottom zone of centrifuge tube. This trend was in accord with the HRTEM results showing the carbonized degree increase from f1 to f17.

![Figure 4. FTIR spectra of original sample and typical fractions (f1, f5, f9, f13 and f17).](image)

**X-ray photoelectron spectroscopy**

X-ray photoelectron spectroscopy (XPS) was used to investigate the surface states of different fractions. The survey spectrum of the CDs before separation (Fig. 5a) showed three typical peaks of C(1s), N(1s), and O(1s). Typical fractions after centrifugation were also tested to identify the variation of their composition. N/C ratio of different fractions (Fig. 5b) decreased as their number increased, indicating that the relative content of N might influence the QY of CDs. It should be noted that the minimum N/C ratio reached 0.16 which is still much higher than that of various N-doped CDs [29].

According to HRTEM images, the very limited lattices we could find were indexed into (100), (020) and (002) facets of graphite carbon (Fig. S1). We can infer that N element did not embed into main structure of C like N-GQD, and the related groups (molecular state) would be released out as the carbonization processing, which might be the one of the main reasons for the origin of high QY. As shown in Fig. 5c, the spectrum of C(1s) in the typical CDs [30] can be deconvoluted into several single peaks corresponding to C≡C/C=≡N (284.3 eV), C-C (284.9 eV), C-N (285.7 eV), C-O (286.5 eV), C=O (288.2 eV) and O-C=O (289.2 eV), which were consistent with the FTIR results. Three typical fractions (f1, f9 and f17) were chosen to provide direct comparison. It is obvious that sp² graphitic C (284.3 eV) increased as the fraction number increased, while other functional
groups such as C-N (285.7 eV), C-O (286.5 eV), C=O (288.2 eV) and COOH (289.2 eV) decreased, confirming the increase of carbonization degree and corresponding decrease of functional groups on the surface of CDs, which was also in accord with the result of HRTEM images (Fig. 3 and Fig. S8). While according to N1s spectrum (Fig. 5d) [31], there was no apparent difference of N species for the three fractions as C-N-C (399.5 eV), N-C3 (400.6 eV), and N-H (401.5 eV), further verifying that there was no change for the existence of N but only amount decrease (Fig. 5b). The FTIR and XPS results also demonstrated that the trend of the amount of surface functional groups was the same as that of optical properties and hydrophilicity, which was the basis of the separation (Fig. 2c).

**Figure 5.** (a) Survey spectrum of XPS measurement of CDs. (b) Experimental results and fitted curve of N/C ratio of separated fractions. (c) C1s and (d) N1s spectra of three typical fractions: f1, f9 and f17.

**Separation mechanism**

Given the close relationship between carbonization degree, morphology and size based on the centrifugation, the separation mechanism of CDs is expected to get better understanding the properties of CDs, especially the most interesting photoluminescence properties. Unlike the CDs obtained from physical synthesis methods, in this study, the CDs were prepared by hydrothermal treatment of solution of citric acid and ethylenediamine, resulting in abundant surface hydrophilic groups (-OH, -COOH, -NH2 and so on) which might form strong hydrogen bonding with solvent such as water. H2O/ethanol solutions were used as the gradient media for the separation zone, as mentioned above, which provided not only a relative lower density gradient than pure water but also a hydrophilicity gradient to weaken the strong interactions between CDs and water. Moreover, after treatment with acetone, the formed tiny clusters (may be coiled structures) other than individual CDs as the target sample would better benefit the sedimentation during the centrifugation. The surface group types for each fraction were essentially the same owing to the same chemical environment in one batch, but the surface group amount of each fraction was different, which played a dominant role in the separation. Specifically, the lower carbonized CDs had more hydrophilic groups on their surface, thereby, would be more affected by hydrophilicity. In this regard, when the tiny clusters moved downward to the gradient medium, fractions with strongest hydrophilicity (fish scale-like polymer species) first tended to drift away from the clusters (de-cluster) and dispersed in the top medium layer. As the centrifugation continued, the rest clusters moved to next layer conducting similar de-clustering behavior. For the bottom fractions (from f15 to f21), they were more like solid carbon particles with few surface groups, and the corresponding separation process was mainly determined by the sedimentation rate difference in density gradient. It should be noted that there was no obvious Raman peaks of typical carbon vibration (Fig. S11 in the ESM) even for the highly carbonized fractions (f17), indicating the poor crystallinity of cores of as-prepared CDs. Therefore, the results verified our assumption about the mechanism of hydrophilicity gradient separation (Fig. 2c). As demonstrated in afore-mentioned control experiments, CDs are almost non-sedimental owing to the abundant hydrophilic surface chemical groups. We tried to solve this problem by pre-aggregating individual CDs into clusters as the separating targets, and realized the separation by the subsequent irreversible dissociation. The form of clusters made the sedimentation realizable. The four layered ethanol/water solutions provided not only the theoretical proper density but more importantly the hydrophilicity gradient for the separation. The pre-aggregated clusters underwent a settlement process accompanied by hydrophilicity controlled
de-clustering during the centrifugation, and the separation effect was strongly dependent on the compatibility between hydrophilicity of CDs and hydroxyl ratio of mixed solvents. The CDs basically stopped sedimentation as long as they met the compatible media even with elongated centrifugation time.

**Transient fluorescence spectra**
As a means to study the exciton delocalization, we measured the time-resolved PL decay (Fig. 6) for original CDs and three typical separated fractions (f1, f9 and f17) at the emission peak (440 nm) with excitation at 340 nm. The decay curves were well fitted by two exponents, as listed in Table 1. There was an obvious change (marked as red hollow circle) for the short-lifetime components as the fractions from top to bottom of centrifuge tube (f1-f17), and the long-lifetime components should be attributed to the surface-state emission. In detail, polymer-like f1 had a slowest decay, 5.2 ns and 14.8 ns. The initially carbonized one (f9) followed, showed 4.6 ns and 12.9 ns. While highly carbonized ones (f17) were fastest, 2.27 ns and 12.38 ns. However, the relative obvious change for the short-lifetime components of the three fractions was not that sharp as the size-dependent highly crystalline CdTe or CdSe QDs, which was attributed to the initially populated core-state recombination [32, 33]. From the direct evidence of HRTEM result (Fig. 3 and Fig. S8), f1 possessed highest QY and was polymer-like cluster structure without the absolute sense of “core”. As the increase of the carbonization, namely, the initial carbonization process, there was amorphous or poorly crystallized solid cores formed, and the corresponding QY of the middle fractions decreased dramatically from f5 to f13 (Fig. 2f) owing to the carbonization induced decline of original luminescent molecular-like cores and sequent self-absorption of solid amorphous cores. For the bottom fractions, the QY were relatively stable (Fig. 2f). Consequently, the PL decay measurement indicated that the emission of the as-prepared CDs was more involved with molecular state. The change of the short-lifetime components might be due to the self-absorption induced quenching phenomenon as the carbonization [34], which is a common and serious problem for fluorescent molecules in solid-state aggregation, including carbon-based materials, and the limited change resulted from the limited aggregation degree during the range of several nanometers range (size distribution of CDs). Comparing with the PL decay of original samples, the existence of most CDs in solution was polymer-like cross-linked clusters, such as f1~f5. These results also confirmed Yang et al’s hypothesis [28] of molecular state which determined the optical properties based on a series of investigation including pH-dependent behavior, solvent-dependent behavior, ferric ions induced dynamic quenching and high power UV lamp induced destruction of PL center.

![Figure 6. Transient photoluminescence spectra of original sample and three typical fractions: f1, f9 and f17.](image)

**Table 1. Fitting results of fluorescence lifetime.**

<table>
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<tr>
<th>Sample</th>
<th>(\tau_1) (ns)</th>
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<th>(\tau_2) (ns)</th>
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<tr>
<td>f9</td>
<td>4.60</td>
<td>0.31</td>
<td>12.91</td>
<td>0.69</td>
<td>10.33</td>
</tr>
<tr>
<td>f17</td>
<td>2.27</td>
<td>0.29</td>
<td>12.38</td>
<td>0.71</td>
<td>9.41</td>
</tr>
</tbody>
</table>

**Proposed PL mechanism of the CDs**
Thus, all the above evidence indicated that the photoluminescence of the as-prepared CDs from hydrothermal reaction was mainly due to the molecular state rather than size quantum effect (Fig. 7). The surface chemical groups (molecular state, probably the imide groups) play a dominant role in the optical properties, which would be the reason why most hydrothermally carbonized CDs show blue photoluminescence and those prepared under milder conditions are brighter [10]. At the same time,
the deeply carbonized cores are poorly crystallized, mainly exhibiting light-absorbing properties. During the carbonization process, in its initial stage, more polymer-like species (such as f1) with abundant chemical groups and no carbonized cores are obtained, which exhibited strong fluorescence (high QY). As the carbonization proceeding, there still exist freshly carbonized species on the surface with abundant chemical groups, which similarly show strong fluorescence, but the carbonized cores with relatively weak fluorescence, would absorb the outer components emitted fluorescence, and cause QY decrease. For the highly carbonized dots, the amount of surface chemical groups significantly decreases and the carbonized cores become the major part, which would induce heavy loss of fluorescence from the outer components, and yield lowest QY among the whole fractions. Thus, the “hydrophilicity gradient separation” method provided an effective way to separate non-sedimental species such as CDs, and based on the separation experiments, new light was shed on the comprehensive understanding of optical properties and rational synthetic design of CDs.

**Figure 7.** Scheme of the proposed PL mechanism of the CDs.

### 4 Conclusions

A hydrophilicity gradient ultracentrifuge separation method was established to separate CDs synthesized from citric acid and ethylenediamine. Clusters of CDs were used as the starting state and the hydrophilicity difference of fractions was used to realize the separation based on four water/ethanol ratios-constituted layers gradient. The clustered CDs were successfully de-clustered and separated basically according to carbonization degree difference in the subsequent separation process. The proposed technique offers a general, novel and effective strategy for the separation of non-sedimental species. This work is also important for understanding the photoluminescence mechanism of CDs. After careful analysis on typical fractions in morphology, size, crystallinity, composition, quantum yield and transient photoluminescence, and concluded that the photoluminescence of as-prepared hydrothermally carbonized CDs mainly arose from the radiative recombination of excitons related to the surface chemical groups rather than carbonized core size difference.

### Acknowledgements

This work was supported by NSFC, the Fundamental Research Funds for the Central Universities (YS1406), the National Basic Research Program 973 (2011CBA00503) and Program for Changjiang Scholars and Innovative Research Team in University.

### Electronic Supplementary Material: Supplementary material of additional HRTEM images, separation method studies, size distribution of separated fractions and spectroscopic studies is available in the online version of this article at http://dx.doi.org/10.1007/s12274-****-****.

### References


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